

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:
Hansgeorg Schindler

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For: ARRANGEMENT FOR VISUALIZING
MOLECULES

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PRELIMINARY AMENDMENT

Commissioner for Patents
Washington, D.C. 20231

Sir:

Applicants respectfully submit this Preliminary Amendment in the above-referenced case.

Consideration of this case in view of the amendments made herein is respectfully requested.

AMENDMENT

In the Specification:

Please amend the specification as follows:

At page 1, line 2, please insert the following paragraph:

--This application is a continuation of PCT Application No. PCT/AT99/00257 filed 28 October 1999, which claims priority to Austrian Application No. A 1799/98 filed 28 October 1998.--

In the Claims:

Please cancel claims 1-23, without prejudice or disclaimer.

Please add new claims 24-60 as follows:

--24. An arrangement for visualizing molecules, movements of molecules, interactions between molecules, and molecular processes in a sample by using the single dye tracing (SDT) method, said arrangement comprising:

- at least one source of light for large-area fluorescence excitation via single or multiple photon absorption by marker molecules in said sample;
- a highly sensitive detection and analysis system comprising a charged coupled device (CCD) camera, at least one of the sample, the sample holding means and the detection and analysis system being movable relative to each other during measuring; and
- a control unit for coordinating and synchronizing illumination times and movements of at least one of said sample and said sample holding means with said sample.

25. An arrangement as set forth in claim 24, wherein said interactions between molecules and said molecular processes are interactions between molecules and processes in biological cells.

26. An arrangement as set forth in claim 24, wherein said marker molecules adsorbed via said single or multiple photon absorption on said molecules in said sample are equal marker molecules.

27. An arrangement as set forth in claim 24, wherein said marker molecules adsorbed via said single or multiple photon absorption on said molecules in said sample are different marker molecules.

28. An arrangement as set forth in claim 24, wherein said control unit is further used to coordinate and synchronize wave lengths.

29. An arrangement as set forth in claim 24, wherein said movements of at least one of said sample and said sample holding means controlled by said sample control unit during measuring are lateral movements.

30. An arrangement as set forth in claim 24, wherein said movements of at least one of said sample and said sample holding means controlled by said sample control unit during measuring are vertical movements.

31. An arrangement as set forth in claim 24, wherein said control unit further coordinates and synchronizes positioning and shifting of images to each sample position on a pixel array of said CCD camera.

32. An arrangement as set forth in claim 24, wherein said at least one source of light is a laser.

33. An arrangement as set forth in claim 32, wherein said laser is an acousto-optically switchable laser light.

34. An arrangement as set forth in claim 24, wherein said at least one source of a light is selected from the group consisting of an argon laser, a dye laser and a two-photon fluorescence excitation laser.

35. An arrangement as set forth in claim 24, wherein said control unit further comprises a pulse transmitter and a software for controlling said at least one source of light and said movement of said sample.

36. An arrangement as set forth in claim 24, wherein said CCD camera includes a frame shift mode and a continuous readout mode.

37. An arrangement as set forth in claim 24, further comprising an epifluorescence microscope.

38. An arrangement as set forth in claim 37, wherein said epifluorescence microscope has a collecting efficiency of fluorescence quanta of >3%, at 40- to 100-fold magnification.

39. An arrangement as set forth in claim 24, further comprising N₂ cooling means provided for said CCD camera, said CCD camera having a large pixel array, a conversion of photons into electrons of from 0.8 to 0.9 in the optical range, a readout noise of only a few electrons per pixel at 1 μs/pixel readout speed, and at least one of << 1 dark counts/pixel x s and a lineshift rate of > 3 x 10⁵/s.

40. An arrangement as set forth in claim 39, wherein said large pixel array is a pixel array of ≥ 1340 x 1300.

41. An arrangement as set forth in claim 24, wherein said sample comprises a molecule library prepared by combinatorial chemistry.

42. An arrangement as set forth in claim 24, wherein said sample comprises a plate selected from the group consisting of a multi-well plate, a microtiter plate and a nonotiter plate.

43. An arrangement as set forth in claim 24, wherein said sample holding means is a flowthrough cell.

44. An arrangement as set forth in claim 24, wherein said highly sensitive detection and analysis system comprises a focussing plane movable step-wise along z direction by a piezo element.

45. An arrangement as set forth in claim 37, wherein said epifluorescence microscope has a parallel beam region as said at least one source of light and comprises a galvano-optical mirror in said parallel beam region.

46. A process for visualizing molecules, movements of molecules, interactions between molecules, and molecular processes in a sample by using a single dye tracing (SDT) method, said process comprising:

providing a sample;

providing marker molecules;

labeling certain molecules in said sample with said marker molecules;

providing an arrangement including at least one source of light for large-area fluorescence excitation via single or multiple photon absorption by said marker molecules in said sample, a sample holding means for accommodating said sample, a highly sensitive detection and analysis system including a charged coupled device (CCD) camera, and a control unit for coordinating and synchronizing illumination times and movements of at least one of said sample and said sample holding means with said sample;

introducing said sample with said marker-molecule labeled molecules into said arrangement; and

imaging said sample by means of said CCD camera on a pixel array, at least one of said sample and said detection and analysis system being moved relative to each other by using a frameshift of said CCD camera such that signals of each individual molecule in said sample are collected in the same pixels after conversion into electrons until said signals of said individual molecule exceed a certain minimum signal/noise ratio.

47. A process as set forth in claim 46, wherein said interactions between molecules and said molecular processes in said sample are molecules and processes in biological cells.

48. A process as set forth in claim 46, wherein said relative movement of said sample is controlled according to the frameshift of said CCD camera.

49. A process as set forth in claim 46, wherein said relative movement of said sample in lateral direction is constant and continuous.

50. A process for quasi-simultaneous imaging of fluorescence-labeled molecules in their distribution over entire biological cells and for observing molecular movements and processes by repeating this imaging at temporal intervals by using a single dye tracing (SDT) method, said process comprising:

labeling certain molecules in a biological cell sample with marker molecules;

introducing said sample with said labeled molecules into an arrangement including at least one source of light for large-area fluorescence excitation via single or

multiple photon absorption by said marker molecules in said sample, a sample holding means for accommodating said sample, a highly sensitive detection and analysis system including a charged coupled device (CCD) camera, and a control unit for coordinating and synchronizing illumination times and movements of at least one of said sample and said sample holding means with said sample;

imaging a fluorescence image of said sample for a focussing plane on a pixel array of said CCD camera;

shifting said focussing plane step-wise along direction z through a piezo element, the fluorescent images for each plane being separately arranged on said pixel array, and, after having imaged all the focussing planes;

calculating the image of the fluorescence-labeled molecules in said cells.

51. A process as set forth in claim 50, further comprising repeating said imaging of said focussing planes so as to trace molecular movements and processes by consecutively arranging images of all said focussing planes.

52. A process as set forth in claim 46, wherein said images on said pixel array of said CCD camera are captured at a rate of from 1 to 3 ms per image and with a capacity of up to 300 images per array, with an image size of 80 x 80 pixels.

53. A process as set forth in claim 46, wherein at last two different types of molecules in said sample are labeled by at least two different fluorescence markers.

54. A process as set forth in claim 46, wherein said fluorescence imaging is effected for two orthogonal polarization directions for each fluorescence marker.

55. A process as set forth in claim 54, wherein said fluorescence imaging for said two orthogonal polarization directions is provided by dividing said image into two images with orthogonal polarization direction by using a Wollaston prism and a source of light having a parallel beam region, the Wollaston prism being used in said parallel beam region of said source of light.

56. A process as set forth in claim 46, wherein said sample comprises cells with low autofluorescence.

57. A process as set forth in claim 46, said process being carried out as a high throughput analysis.

58. A process as set forth in claim 46, wherein said sample is a molecule library.

59. A process as set forth in claim 58, wherein said molecule library is prepared by combinatorial chemistry.

60. A process as set forth in claim 46, said process being used for analyzing the interaction of a molecule library with biological cells.--

REMARKS

The specification has been amended to recite the priority data, to cancel claims 1-23 of the PCT application, and to add new claims 24-60. The filing fee has been calculated after amendment of the claims by the preliminary amendment

Should any additional fees under 37 C.F.R. §§ 1.16 to 1.21 be required, the Commissioner is hereby authorized to deduct said fees from Fulbright & Jaworski Deposit Account No. 50-1212/10103495/MBW.

Respectfully submitted,



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